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Nitrogen Fixation Systems in Forest Plantations

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I. INTRODUCTION

The most abundant element in the atmosphere is nitrogen and yet it is very often the limiting element for the growth of most organisms. The N of the atmosphere and the biota are linked principally through a group of micro-organisms that can fix N into forms that are usuable by the remainder of the microbes, plants and animals. Small amounts of N are fixed naturally through such abiotic agents as lightning and combustion. Also man fixes N chemically to form nitrogenous fertilizers.

The fertilizers used, world-wide, in 1980 contained approximately 5.8×10^7 tonnes of N. This constituted approximately 25% of all N inputs to

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biological systems. All other abiotic sources combined, equalled less than an additional input of 10%. Thus, of annual total global inputs of N, more than 65% can be conservatively estimated to come from biological sources.

Extensive forest management or exploitive harvesting of forest resources have usually depended on natural N-fixation and cycling. Frequently this has resulted in slow growth of the trees and long rotations (Skau *et al.*, 1970). For intensive forest management, the amount of fertilizer N and the area fertilized have increased during the last 10–15 years (Bengtson, 1979). They will probably continue to increase as we learn more about which species, at which ages and on which soils, are most likely to give an economic response.

Interest in N dynamics in forest ecosystems has increased over approximately the last decade (Davey and Wollum, 1978, 1979; Farnsworth *et al.*, 1976; Gordon *et al.*, 1979; Jurgensen and Davey, 1970; Skinner, 1976; Wollum and Davey, 1975). Forest growth can be enhanced by additions of N or by increasing the rate of N cycling within the ecosystem. The greatest potential for increased forest productivity lies with enhanced N fixation on forest sites. It has attractiveness because the N is fixed on site by biological entities. Thus it obviates the need for fossil fuel feedstocks for fertilizer manufacturing or for transportation of the fertilizer. Ultimately, however, the N-fixing organisms must compete with the non-N-fixing crop trees for space, water and nutrients other than N. Thus both the biology and the economics of any potential N fixation component of forest management need to be understood and evaluated.

In this chapter, we have briefly reviewed sources of biological N fixation, possible adaptation of some to forest management, constraints in introducing and managing N-fixing biota in plantations and their potential for the future.

II. SURVEY OF DINITROGEN FIXATION

Nitrogen is fixed in many habitats by organisms that vary from aerobes to strict anaerobes and includes saprophytes and photoautotrophs. Some organisms fix N in the free-living condition and others fix N only in association with some other organism. This great diversity assures that some N is likely to be added to essentially any habitat that can support forest growth.

The organisms capable of N fixation are all prokaryotic and the nitrogenase enzyme appears to be similar in them all. The N-fixing process requires high levels of ATP for the reduction of N to ammonia. Nitrogen fixation is not without considerable cost in terms of energy and carbon (C); frequently ranging from 0.3 to 7 mg C/mg N fixed (Bowen, 1978).

A. Non-symbiotic Organisms

(1) Aerobes

Bacteria and blue-green algae occur in this group. The bacteria are found in such genera as *Azotobacter* and *Beijerinckia* and the algae in *Anabaena* and *Nostoc*. Of these, only *Beijerinckia* tolerates the high soil acidity commonly found on forest sites. Blue-green algae were not found in any forest soil more acid than pH 5.5 (Jurgensen and Davey, 1968). Additionally, being phototrophic, they would not be very active under the closed canopy of a forest. They may be ecologically important in the recolonization of severely burned forest sites, and they are found in forest nursery soils.

(2) Facultative anaerobes and microaerophiles

These are all bacteria but some are saprophytic and others are phototrophic. The saprophytes include: *Bacillus, Klebsiella, Thiobacillus* and *Azospirillum*. All are aerobic except when fixing N and all are known to occur in forest soils. The phototrophs include only a few genera. They are represented by *Rhodospirillum* and if present, they apparently play minor roles in forest soils.

(3) Strict anaerobes

As in the above group these are all bacteria, some of which are saprophytes and others are phototrophs. The saprophytes include the genera *Clostridium* and *Desulfovibrio*. The genus *Clostridium*, like the genus *Bacillus*, produces endospores. Thus they are able to survive severe environmental conditions. These two genera also tolerate acidity down to pH 2 (Buchanan and Gibbons, 1974). They are found in many forest soils and may be responsible for a major portion of the N that is fixed in forest soils by free-living organisms (Jurgensen and Davey, 1970, 1971). The anaerobic phototrophs include the genus *Chromatium*, which is found primarily in aquatic environments.

B. Symbiotic and Associative Organisms

(1) Leguminous plants

The best-known and most-studied N-fixation systems are those between leguminous plants and bacteria in the genus *Rhizobium*. This genus is divided into six species and one group, depending on the plant host (Postgate, 1978).

The bacteria may be further divided serologically, allowing workers to trace the fate of specific strains in soil (Dudman, 1977).

Many leguminous plants are found in forest ecosystems. Their diversity is reflected by a survey of 100 hectares of a pine-hardwood forest in the eastern United States, in which 21 species of understory native leguminous plants were found (Krochmal and Kologiski, 1974). Unfortunately, nodulation was not recorded and thus their potential for symbiotic N-fixation is unknown. In fact only about 20% of the approximately 14 000 known leguminous plant species have been checked for nodules. However about 90% of those investigated have been found to be nodulated. Tree-form leguminous plants are components of the forest from the cool temperate regions to the tropics.

(2) Actinorhizal Plants

Most of the plants in this group are woody shrubs but a few are important timber trees. This group of plants (all angiosperms, but including at least 10 families, 15 genera, and 113 species: Youngberg and Wollum, 1970) possess root nodules which are inhabited by N-fixing actinomycetes in the genus *Frankia*. Because of great difficulty in working with this group of endophytes, their taxonomic position has long been in question. It now appears that the genus *Frankia* is their appropriate designation (Lechevalier and Lechevalier, 1979). However, species names are not yet accepted.

Tree-form actinorhizal plants occur in two genera: *Alnus* and *Casuarina*. *Casuarina* is found only in the sub-tropics and tropics (see Chapter 18), while most of the other actinorhizal plants are found from warm temperate to boreal habitats.

Advances in our understanding of this important group of N-fixing forest plants have come rapidly. A milestone was reached with the publication of the work of Callaham *et al.* (1978) demonstrating the isolation and culturing of the endophyte of *Comptonia peregrina* followed by successful inoculation and nodulation of the host and reisolation of the endophyte.

(3) Non-nodulated plants with root- and leaf-associated bacteria

Certain tropical grasses have N-fixing bacteria intimately associated with their roots. Principally these include *Azospirillum lipoferum* in a zone beneath the root epidermis of *Digitaria decumbens* (Dobereiner and Day, 1974) and *Azotobacter paspali* in a mucilaginous sheath around the roots of *Paspalum notatum* (Dobereiner and Campelo, 1971). *Azospirillum* has been isolated from some temperate soils. The optimum temperature for N fixation has been reported to be above 36°C (Dobereiner and Day, 1974).

Bacteria in the genus *Beijerinckia* fix N in the basal sheath of the leaves of the tropical grass *Tripsacum laxum* (Ruinen, 1970). Whether any of these associations might be useful in the establishment of forest plantations in tropical regions remains to be determined.

The literature contains reports of various leaf-microbe symbioses wherein the host plant is stimulated. Nitrogen fixation was assumed to be the cause, but acetylene reduction assays have failed to confirm N fixation, and the growth stimulation is now thought to be the result of the production of growth hormones (Postgate, 1978).

(4) Blue-green algae-associated plants

Nitrogen-fixing, blue-green algae form symbiotic associations with several lower plants. Certain lichens are able to colonize inhospitable bare areas because of a N-fixing phycobiont. Growth is usually very slow, but soil formation is initiated and N does accumulate. The blue-green alga *Nostoc* fixes N when in association with the thalli of liverworts and the N is incorporated into the host plant. The very small aquatic pteri~~o~~dophyte *Azolla* harbours the blue-green alga *Anabaena* in a cavity at the base of its fronds. This system has been found to be very effective in providing N to paddy rice. Some cycads have coralloid roots inhabited by *Nostoc*. In this environment the alga does not need light for N fixation. None of these systems are likely to be important in forest plantations.

(5) Other plant-microbe associations

Two other associations are worthy of note because of their uniqueness and potential. One subtropical angiospermous tree in the genus *Gunnera* harbours the N-fixing blue-green alga *Nostoc* in nodules at the base of the leaf. Fixed N is incorporated in the tree. Bacteria of the genus *Rhizobium* have been found to fix N in nodules on the roots of a tropical tree in the genus *Parasponia* (Akkermans *et al.*, 1978), which is not leguminous. These observations may have important implications for intensive forest management in the subtropics and tropics.

III. ASSESSMENT OF DINITROGEN FIXATION IN FORESTRY

Estimates of amounts and rates of N fixation were traditionally hampered for lack of suitable methods, but the availability of ^{15}N for use in such studies increased the amount and precision of our knowledge. However, ^{15}N is expensive, and since it is a stable isotope, it requires a mass spectrograph for its detection. These two factors inhibited rapid progress in the field. With the development of a presumptive test for nitrogenase activity (N-fixation), through acetylene reduction to ethylene and its determination by gas chromatography (Hardy *et al.*, 1973), progress accelerated greatly. The acetylene reduction technique has proved to be both rapid and dependable for identifying potential sources of biological N-fixation. However, quantification of amounts of N fixed still require the use of ^{15}N enrichment or measurement of absolute gains of total N in the organism or ecosystem.

A. Amounts of Nitrogen Fixed

(1) Non-symbiotic organisms

Despite their obvious scientific importance and their long-term ecological significance, the free-living organisms do not appear to contribute much to the N economy of forested lands. The highest estimate noted was 595 kg N ha^{-1} yr^{-1} for a 3-year period in regenerating lowland humid bush in Nigeria (Jaiyebo and Moore, 1963). These authors also reported gains of 90 kg N ha^{-1} yr^{-1} under the grass genus *Cynodon*. Other early estimates of N fixed, predominantly by non-symbiotic organisms, in the tropics in kg N ha^{-1} yr^{-1} were: lowland forest, up to 165; rain forest, 73; highland forest, 45; and savannah, 17 (Greenland and Nye, 1959). However, these earlier N-fixation rates were optimistically high, and current estimates of non-symbiotic N fixation in forest ecosystems have decreased dramatically. Values in kg N ha^{-1} yr^{-1} have ranged mostly from 0.06 to 0.94, with nearly all values being less than 10 (Hardy *et al.*, 1973; Jorgensen, 1975; Larson *et al.*, 1978; Sucoff, 1979). In his excellent essay on N fixation, Postgate (1978, page 33) put the subject in proper perspective:

> It is important to be clear that free-living nitrogen-fixing microbes, at least when in the free-living state, are rarely of serious importance in the terrestrial nitrogen economy.

Apparently the proviso ". . . when in the free-living state . . ." is to clearly exclude those non-symbiotic organisms which do fix more significant amounts of N when in very close association with some higher plant (Dart and Day, 1975; Dobereiner and Day, 1974; Ruinen, 1970).

The non-symbiotic, but associative organisms have been responsible for some moderately high documented fixation rates. However, the majority of the reports are below 30 kg N ha^{-1} yr^{-1} and most are below 5. In a review article, Evans and Barber (1977) cite their own work with *Zea mays* and *Spirillum* (*Azospirillum*) in which under ideal conditions in the greenhouse, N was fixed at a rate of 0.7 kg ha^{-1} day^{-1}. Despite this impressive rate, they note that the associative systems are very unpredictable because the nitrogenase enzyme in the system is very susceptible to oxygen damage. They suggest that symbiotic systems are currently more likely to provide significant amounts of fixed N.

(2) Symbiotic systems

Reports such as that of Denison (1979), showing that lichens inhabit the crowns of old-growth *Pseudotsuga menziesii* (Douglas fir) and fix N symbiotically at rates of 3 to 4 kg ha^{-1} yr^{-1}, do exist, but from the standpoint of the management of forest plantations only two symbiotic systems need to be considered. They involve leguminous and actinorhizal plants and their respective N-fixing endophytes.

The agricultural literature is almost entirely devoted to leguminous plants while the forestry literature contains both, but with a definite emphasis on the actinorhizal plants; especially members of the genus *Alnus*. The amount of N reported to be fixed has varied considerably. The lowest values have been reported for *Myrica cerifera* (wax myrtle). The first estimate for this species was 15 kg N ha^{-1} yr^{-1} (Silver and Mague, 1970). In a more recent study, a regression equation was developed to predict N fixation as a function of the percent ground cover of the species (Fisher *et al.*, 1979). Although the average N fixation rate was found to be 9 kg ha^{-1} yr^{-1}, the ground cover was rather sparse. At 100% ground cover, the predicted rate was 113 kg N ha^{-1} yr^{-1}. *Myrica cerifera* is a woody shrub found frequently in the pine forests in the southeastern United States. It is large enough to compete with the pines for growing space, thus the value of the added N must be calculated on the basis of fewer, but larger crop trees than would be the case in the absence of this N-fixing plant.

High rates of N fixation in forest ecosystems have been reported for the genus *Alnus*; particularly the timber tree *Alnus rubra* (red alder). The highest rate noted for that species has been 320 kg N ha^{-1} yr^{-1} (Newton *et al.*, 1968) while others report values varying from 80 to 200 kg N ha^{-1} yr^{-1} (Gessel and Turner, 1974). In a detailed study of N accretion in the soil (20 cm depth) and forest floor, Bormann and De Bell (1981) reported that in stands of *A. rubra* up to 41 years of age, the soil N increased linearly at 35 kg ha^{-1} yr^{-1}. The forest floor N accumulated rapidly to age 5 and then became linear at 15 kg ha^{-1}. These are conservative estimates since no account was made of N retained in the trees or other vegetation or of N lost from the site.

Nitrogen fixation by *Alnus incana* averaged 156 kg N ha^{-1} yr^{-1} up to 20 years of age in a river flood plain in Alaska (Van Cleve *et al.*, 1971). They reported, however, that for the first five years the net rate was 362 kg N ha^{-1} yr^{-1}.

Alnus rubra has been grown in mixture with other crop trees. *Pseudotsuga menziesii* was grown in a pure stand and mixed with *A. rubra*: at 27 years of age, the tree volume was $6.4 \times 10^3 m^3 ha^{-1}$ in the mixed stand, and $4.8 \times 10^3 m^3 ha^{-1}$ in the pure *P. menziesii* stand (Tarrant, 1961). At 30 years of age, the N economy of the sites was evaluated; it was determined that N had accrued in the soil where *A. rubra* was present at the rate of 40 kg N ha^{-1} yr^{-1} (Tarrant and Miller, 1963). In another study of pure and mixed species, *A. rubra* and *Populus trichocarpa* (California poplar, black cottonwood) were grown alone and in mixed stands (De Bell and Radwan, 1979). In the mixed stand, N fixation was reported to be 32 kg ha^{-1} yr^{-1} and in the pure *A. rubra* stand it was 80 kg ha^{-1} yr^{-1}. Biomass production was 9.8 t ha^{-1} yr^{-1} in the mixed stand and 6.3 and 5.7 t ha^{-1} yr^{-1} in the pure alder and cottonwood stands, respectively.

Nodulation, above-ground biomass production, and N accretion were studied over a 15-year-period in a *Ceanothus velutinus* stand which followed a *P. menziesii* clearcut (Youngberg *et al.*, 1979). Over the 15-year-period, N accretion averaged 84 kg ha^{-1} yr^{-1} and *P. menziesii* seedling stocking was 48% higher beneath the *C. velutinus* than in the open. In another study, Youngberg and Wollum (1976) measured N accretion associated with *C. velutinus* on *Pinus ponderosa* (ponderosa pine) and *Pseudotsuga menziesii* sites over a ten-year-period. Although nodulation was greater on the *P. ponderosa* site, the N accretion was greater on the *P. menziesii* site (71.5 kg N ha^{-1} yr^{-1} *vs* 108.0 kg N ha^{-1} yr^{-1}, respectively). The authors attributed the difference to better moisture relations on the *P. menziesii* site which receives 1750 mm of precipitation annually compared to 460 mm on the *P. ponderosa* site.

All of the above citations have been intentionally restricted to actinorhizal plants. Amounts reported fixed by leguminous plants in forest ecosystems are within the same general ranges although frequently lower, and certainly far below the maximum values reported for agricultural systems. This is probably because of sensitivity of the leguminous plants to soil acidity and associated high levels of exchangeable aluminium and low P availability. Leguminous plants predominate in the tropics, although the tropical forms of the actinorhizal plants of *Casuarina Alnus* and *Myrica* deserve further investigation.

B. Dynamics

Questions arise not only about the rate of N fixation, but also about the time course of the fixation, the release of fixed N to the soil and higher plants, and the overall gains of N by the site. When the N-fixing system includes a timber tree such as species of *Alnus, Casuarina* or *Robinia* the benefit is immediate. However, in many cases there is a delay since the principal crop tree and the plant involved in the N fixation are different.

There appears to be a rapid accumulation of N for the first several years (up to 5 in many systems) when the rate of fixation is high (Van Cleve *et al.*, 1971). This is then followed by a prolonged period of steady N gains if the N-fixing plant remains in the canopy. This is true whether the N-fixing plant is a tree or a dominant of the lesser vegetation. In other situations where the principal crop trees capture the site, the N-fixing plant is slowly shaded out and N gains cease (Gadgil, 1971c; Sprent and Silvester, 1973). This does not imply, however, that the benefit to the site has ended.

Nitrogen that has been incorporated in the forest floor through biological fixation continues to mineralize over a period of years, the length of which varies with the amount and nature of the residues, the climate (moisture and temperature, principally) and the characteristics of the principal crop trees.

The magnitude of the total amount of N fixed over a crop rotation was provided by Zavitkovski and Newton (1971). They estimated that in a stand of *A. rubra*, over a 50-year-period, some 10 t N ha^{-1} would be contributed to the site. In addition, the total litterfall would exceed 300 t ha^{-1}, approximately 280 t of which would be broken down and incorporated with the mineral soil. Zavitkovski and Newton (1971) speculated that such incorporation would create favourable physical, chemical and biological conditions for tree growth. There is no doubt that although some N is lost from the site through leaching and denitrification, probably very little is lost by erosion. Thus the overall gains to the site will be something less than the amount fixed but will still be important to site quality. The situation described here may represent a near maximum value of N accretion and serves to emphasize the potential of symbiotic N fixation for use in intensive plantation forest management.

C. Alternative Forest Management Applications

Several alternatives exist for use of N-fixing plants in plantation forestry. This is true both as to the plant species which may be used (Gordon and Dawson, 1979) and the timing involved. The three basic approaches include: (1) interplanting N-fixing trees with non-N-fixing trees; (2) underplanting crop trees with a N-fixing ground cover; and (3) alternating crops of N-fixing plants and non-N-fixing crop trees.

(1) Interplanting

One of the problems of interplanting is that the N-fixing species often outgrows and suppresses the non-fixing species. An interesting example of how this may be overcome involves the mixture of *A. rubra* with *P. menziesii* at Wind River, Washington (Tarrant, 1961). The provenance of *A. rubra* planted was not well adapted to the harsh winters of the site and was injured several times over the life of the plantation. Consequently the *P. menziesii* maintained dominance and responded very significantly to the N contribution from the *A. rubra*.

Elaeagnus umbellata was interplanted with the very valuable *Juglans nigra* (black walnut) at locations including both upland and bottomland sites (Funk *et al.*, 1979). Growth stimulation of *J. nigra* was evident at age 4 and it became taller than the *E. umbellata* at age 6 on the bottomland and at age 8 on the upland sites. After 9 years, the *J. nigra* in the mixed planting were 82–134% taller than those in pure stands.

For very short rotations, coppiced growth of mixtures of *Populus trichocarpa* and *A. rubra* shows promise (De Bell and Radwan, 1979). Annual dry matter production was greater in the mixed planting than from either species alone. The *P. trichocarpa* has the genetic potential to grow very rapidly, but frequently lacks sufficient N to express this capability. Twigs of *P. trichocarpa* from mixed stands contained 18% more N than twigs from pure *P. trichocarpa* stands. There was 9 and 23% more N in the soil under mixed stands and pure *A. rubra* than under pure *P. trichocarpa* stands. This amounted to 855 ppm N in soil beneath *P. trichocarpa*, 935 ppm beneath mixed stands, and 1055 ppm beneath *A. rubra*.

The leguminous tree, *Robinia pseudoacacia* (black locust) has been interplanted with other forest species. Problems of susceptibility to insects and disease have made such tests mostly inconclusive, although some N is fixed. This species deserves attention through genetic selection for insect and disease resistance or through their cultural control.

Leguminous trees offer great potential for use in tropical forestry, but this resource has hardly been tapped (National Academy of Science of the United States, 1979). Trees in the genera *Acacia*, *Acrocarpus*, *Albizia*, *Leucaena*, *Mimosa* and *Sesbania* show promise for interplanting with more valuable tropical timber species. Leguminous trees in the genera *Dalbergia*, *Intsia*, *Pericopsis* and *Pterocarpus* are valuable timber trees in their own right, but might be profitably mixed with other species. The species *Leucaena leucocephala* includes varieties that range in form from shrub to true tree (e.g. variety K-8). It has potential for interplanting or underplanting, depending on the variety selected. The tropical actinorhizal tree *Casuarina equisetifolia* (horsetail oak, coastal sheoak) has not been reported to have been successfully interplanted with other trees. Its rapid height growth and crown spread make it a vigorous competitor on many sites. The genus contains about 80 species, and some less aggressive species would be useful for this purpose.

(2) Underplanting

The underplanting of N-fixing ground cover plants in a forest plantation may have several advantages in addition to the added N (Haines *et al.*, 1978). Development of a good ground cover may reduce erosion and eliminate the need for cultivation of young plantations. The best species with *Platanus occidentalis* were *Trifolium subterraneum* and *T. incarnatum* which are cool season plants. They grew during the fall and spring and did not compete for moisture and nutrients during the principal period of tree growth. The mat of dead plants controlled the growth of weeds, especially grasses, during the summer. They have the added advantages of being low growing and non-vining in growth habit which lessens early shading effects on the crop tree. After four years, the *P. occidentalis* with the *Trifolium* spp. were on the average 7.3 m tall as compared to 3.0 m for *P. occidentalis* without an under-planting of *Trifolium* spp. Their respective volumes were 53.9 and 14.5 $m^3 ha^{-1}$.

In the above case, the N-fixing plants were seeded in after the trees were established, but other planting sequences may also work well (Gadgil, 1971a, b, c). On N deficient, phosphorus sufficient sand dunes in New Zealand, stabilization by *Pinus radiata* is a three-step process. First, grass is established, then lupins are established in the grass and allowed to grow, preferably for three years (Gadgil, 1971c). Following this, some lupins are crushed by tractor and pine seedlings are planted among the crushed lupins, but protected by the shade of the undamaged ones. After thinning of the plantation at 10–12 and 18–22 years, there is rapid regrowth of lupins and apparently the addition of more N to the site.

The suitability of herbaceous leguminous plants for N fixation in *Pinus taeda* (loblolly pine) plantations is being evaluated in the southeastern United States. Four *Trifolium* spp. which received a commercial *Rhizobium* inoculum have been evaluated after four years in a *P. taeda* plantation (H. L. Vanderveer, N. C. State Univ., unpublished). The *Trifolium* spp. were seeded with the pine seedlings with or without a fertilizer + lime treatment. Two of the *Trifolium* spp. died soon after establishment, but *T. subterraneum* and *T. vesiculosum* persisted and reseeded each year. Neither the *Trifolium* spp. alone, nor the fertilizer + lime treatment alone strongly influenced tree growth (it should be noted that since this was a test of leguminous plants, the fertilizer + lime treatment included only 28 kg N ha^{-1}). In the combination plots, after four years the tree N content was approximately tripled

(31 *vs* 10 kg N ha^{-1}) and the tree biomass more than doubled (1965 *vs* 777 kg ha^{-1}). Much remains to be learned about systems similar to this, but these preliminary results are encouraging.

(3) Rotational planting

Rather than interplanting crop trees with N-fixing trees or ground cover, it should be possible to carry out a crop rotation such that the site would first be ameliorated by growing an N-fixing species. Then, if it were an income-producing species, it could be profitably harvested. Finally, the crop tree could be grown on the N-enriched site. No actual tests of such crop rotations are known to the authors, but Atkinson *et al.* (1979) proposed six different rotations involving *Pseudotsuga menziesii* and *A. rubra*. After establishing a number of assumptions, they made an economic analysis of each system. All rotations were profitable, but under the assumptions made, continuous *P. menziesii* was the most profitable. The authors cautioned that several of the asumptions were quite uncertain and suggested that the more promising rotations be tested. The problem was an anticipated low value for the *Alnus* wood in comparison to that of the conifer.

The economics of such crop rotations might be more favourable for sites that have been drastically disturbed, such as mine spoils. On such areas, common crop trees frequently will not grow at an acceptable rate because of N deficiency, low soil organic matter content, and adverse soil physical factors. The growth of a N-fixing crop prior to the desired tree crop may be essential in order to grow the principal crop.

IV. POTENTIALS FOR THE FUTURE

The foregoing sections of this chapter have shown that there has been only very limited intentional use of any N fixation system in the intensive management of forest plantations. The reader should not expect that all sites and situations are suitable, but neither should the idea be abandoned. We conclude that there is good potential for eventual success with symbiotic N fixation in plantation forest management. This chapter is brought to a close by looking first at presently existing constraints and then to the future.

A. Constraints

Factors that can limit or prevent success in the use of symbiotic N fixation involve the endophyte, the host, or the site. These are interconnected in the forest but they need to be studied separately at first and then in combination.

Methods for the isolation and study of *Rhizobium* have been available for about half a century, whereas those for studying *Frankia* have existed for less than five years (Baker and Torrey, 1979; Berry and Torrey, 1979; Callaham *et al.*, 1978; Lalonde and Calvert, 1979). Since that major hurdle has been cleared we can expect progress in knowledge of the ecology of *Frankia*. Techniques which have been developed for use with *Rhizobium* such as density gradient separation from soil (Wollum and Miller, 1980); fluorescent antibody and ELISA (enzyme linked immuno-sorbent assay) techniques for strain identification (Jones and Morley, 1981; Roughley *et al.*, 1976) may soon be adapted to work with *Frankia* as well. From studies using such techniques will come information on strain variability for efficiency of N fixation and ecological competence.

Although commercial inoculum is available for most agriculturally important leguminous plants, the strains of *Rhizobium* contained in most inocula are not well-adapted to harsh forest sites. Commercial inoculum for actinorhizal plants is non-existent. Thus unless there are infective, effective, efficient strains of *Rhizobium* or *Frankia* in forest soils of interest, it will be necessary for such inocula to be developed. Strain selection for forest sites for *Rhizobium* has been started (Cañizo *et al.*, 1978; Thornton, 1981); much remains to be done.

Selection of varieties or even provenances of leguminous or actinorhizal hosts which are adapted to forest sites has been limited. So far, most of the screening has been with leguminous plant species and cultivars that have been developed for agricultural rather than silvicultural conditions. If adapted hosts are found this way, one other limiting factor may be avoided: a source of seed. If foresters must use native N-fixing plants, they will then be forced to provide for the production of seed. This certainly is not an impossibility, but it does add to the complexity and time required for practical application.

Limiting factors of both abiotic and biotic origin occur on the site. Acidity and exchangeable aluminium are important (Bromfield and Jones, 1980; Keyser and Munns, 1979; Thornton, 1981). Also, soil fertility (Hagedorn, 1979), moisture availability (Mahler and Wollum, 1980) and temperature tolerance (Lindermann and Ham, 1979; Munevar and Wollum, 1981) are important and candidate endophytes must be screened against them.

The biotic factors which can be limiting involve competition between endophytes introduced in inoculum, with indigenous members of the same species (Boonkerd *et al.*, 1978; Holland, 1970; Winarno and Lie, 1979). There is also competition between endophytes and rhizosphere inhabitants of the host (Reyes and Schmidt, 1979).

A final limiting factor can be the second endophyte which must share the host: the mycorrhizal fungus. For example, with *Alnus*, phosphorus nutrition of the host is very important in energy (ATP) availability for N fixation and the mycorrhizal fungus can have a significant effect on its uptake (Mejstrik and Beneke, 1969). Two suggestions for simultaneous selection of both endophytes have been published (Hall *et al.*, 1979; Trappe, 1979).

B. Outlook for the Future

The outlook for future exploitation of N fixation in management of forest plantations is bright. We can be fairly certain that it will be highly beneficial on sites that are very N-deficient (Gadgil, 1971a) and would not be a sound investment on N-rich sites. By far the majority of the forest lands fall between these extremes, with most being responsive to additional available N. Both the biology and the economics of various possible plant combinations need to be investigated (Atkinson *et al.*, 1979).

Since the evidence strongly indicates that the symbiotic systems are most likely to be successful, certain requirements and limitations are automatically set. They include that we work almost exclusively with leguminous or actinorhizal plants. The exciting caveat "almost" refers to possible exceptions such as the successful association of *Rhizobium* with a non-leguminous tree in the *Ulmaceae* (Akkermans *et al.*, 1978; Trinick, 1973). Perhaps in the long term, genetic engineering will make such exceptions commonplace, but for the short term we should be alert to such possibilities while investing the majority of our resources on known systems.

A second requirement-limitation in working with symbiotic systems is for light for the host plant. If the host plant is a tree, this requirement may be met throughout the life of the stand. If the host plant is a member of the understory, N accretion will likely cease when the canopy closes. The benefit may continue for several more years however (Bengtson and Mays, 1978), and may recur following heavy thinning (Gadgil, 1971a). The possibility of rotating crops could allow herbaceous or shrubby plants to receive full sunlight for a prolonged period.

A third point is that we should work simultaneously with two endophytes: the N-fixing *Rhizobium* or *Frankia* and the mycorrhizal fungus. A plea for work on both endophytes was made by J. M. Trappe (1979) in his paper sub-titled "A quest in need of questers". The potential benefits from properly matching site, host and two endophytes are considerable.

The preponderance of research conducted to date in this field has dealt with temperate forests. This information is valuable but must be extrapolated with caution to the tropics. Since there is a greatly expanding use of plantations in tropical silviculture, much new work is needed, built upon existing knowledge but devoted to tropical systems.

Man has known of the existence of the element N for slightly over 200 years. It was discovered by Daniel Rutherford in 1772. The value of N-fixing plants, however, has been known for much longer. We close this chapter with a quote from William Browne, published some 159 years before the work of Rutherford (Browne, 1613). It both tells the history and sets the stage for the future in this important field in one brief, rhyming couplet:

> The alder whose fat shadow nourisheth—
> Each plant set neere to him long fluorisheth.

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